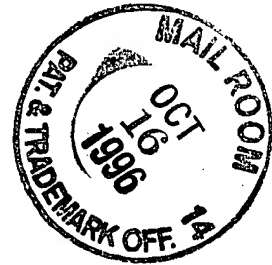


IN THE UNITED STATES PATENT OFFICE

Application Serial No. 07/675,908

Filed: July 3, 1991

Applicants: Dr. Rudolf Falk
Dr. Samuel S. Asculai
(Now assigned to
Hyal Pharmaceutical Corporation)



Title: THE USE OF HYALURONIC ACID OR ITS
DERIVATIVES TO ENHANCE DELIVERY
OF ANTINEOPLASTIC AGENTS

Inventors: Dr. Rudolf Falk,
Dr. Samuel S. Asculai

Examiner: Dr. Jacqueline Krikorian Ph.D. (formerly Dr. Stephen Martin, Ph.D.)

Group Art Unit: 1306 Extended Due Date: September 5, 1996

The Commissioner of Patents
UNITED STATES PATENT OFFICE
2011 Jefferson Davis Highway
Crystal Plaza 2, Room 1E03
Arlington, Virginia
U.S.A. 22202

**DECLARATION OF JOSEPH ROBERT EMMOTT FRASER
under § 1.132**

I, JOSEPH ROBERT EMMOTT FRASER, M.D., make oath and say as follows:

1. I graduated as a legally qualified medical practitioner from the University of Melbourne (M.B., B.S., a six-year course), and subsequently, specialized in Cardiology while taking out the M.D. in 1954, (a higher degree in this University) and qualifying for college memberships as an internist and was later elected to Fellowship of both. The reader will note that M.D. is a higher doctorate here as

A handwritten signature in black ink, appearing to be "J.R. Fraser".

in the U.K. and Scandinavia. The Australasian and London Colleges of Physicians are Colleges of internal medicine.

As a Cardiologist, with an interest in the mechanisms of rheumatic fever and related joint diseases, I devised a new method for the culture of synovial cells which lined the joints and then realized, having regard to the published literature at the time and the available knowledge, that very little was known about the metabolism of hyaluronic acid (HA), the distinctive component of joint fluid later designated hyaluronan. I was able to use these cultures to synthesize the substance and gradually developed methods to produce highly purified radioactively labelled hyaluronan of naturally occurring very high molecular weight. At the time, this seemed to provide the only means to solve some of the fundamental questions concerning its turnover in the body.

2. (a) Now shown to me and marked as *Exhibit 1* to this my Declaration is my Curriculum Vitae. The reader will note my extensive involvement as a Doctor, Cardiologist, and practicing physician. The reader will also note the number of papers which I have written particularly, starting about 1961, relating to hyaluronan. The reader will note that from 1961 to 1988/89, I have been author or co-author of more than in excess of seventy (70) papers and from 1990 to the present. I have been author or co-author of an additional thirty (30) papers dealing with hyaluronic acid. See, for example, publication #132 entitled, "'Hyaluronan'. In *Extracellular Matrix*, Vol. 2, Ch. 5, Pp. 1410199. Ed. W.D. Comper. Harwood Academic Publishers, New York & London. 1996" in my bibliography. In this time, I have taken a close interest in all aspects of the sources, structure and functions of hyaluronic acid and its transport and degradation, especially in the living body.



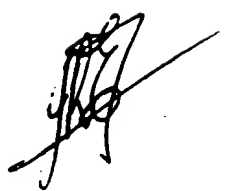
(b) I have, as an expert in respect of hyaluronan, assisted Hyal Pharmaceutical Corporation, the Assignee of the above-identified patent application in the U.S. Patent Office. As a consultant, I was involved in advising Hyal Pharmaceutical Corporation in all aspects of hyaluronan and have carried out research and development and testing for Hyal Pharmaceutical Corporation. I would not, however, let my acting as a Consultant for Hyal Pharmaceutical Corporation or for anyone, interfere with or cloud my professional objectivity and responsibilities in rendering any opinion for any person.

3. In 1973, I showed that hyaluronan escaped, at least in part, through the lymphatic drainage of the joint and that hyaluronan disappeared very quickly from the blood stream, matters which had not been previously studied. To this time, there had been no recorded evidence that HA moved out of the tissues at all. Its blood levels were known to be very low and extensive work to detect hyaluronidase in all kinds of tissue implied that people thought it was turned over "on the spot", probably due to its enormous molecular dimensions.

4. During the formulation of plans to extend these findings, I discovered that Professor Torvard Laurent, who was in Melbourne on sabbatical leave in 1979-80, had just proven by direct measurement that significant amounts of HA passed from the tissues to the blood stream through the lymphatic channels. I arranged to spend my study leave in his Institute to carry out my plans to use radioactive HA to trace its disposal in the living body, and have been a visiting scientist in his Institute on many occasions since. We found we shared similar views and have subsequently collaborated (with Ulla Laurent and others) to

1) show that hyaluronan is eliminated almost entirely in the body by cellular uptake and metabolic degradation,

2) identify all the major cell types and sites where this occurs after it leaves the tissues,



3) demonstrate all the chemical steps in its complete degradation in the body, and

4) determine its rate of turnover in joints, skin, and other sites.

5. From my previous work with respect to hyaluronan, we deduced and then demonstrated that lymph nodes are a most important participant in hyaluronan's metabolic elimination, incidentally adding a new category to the recognized functions of lymph nodes. In brief, we established clearly for the first time that hyaluronan (HA) does move through the body and identified the channels through which it passes.

6. I have also conducted research on the viscous behaviour of hyaluronan and its interactions with proteins. As a clinician, I have followed closely the development of hyaluronan as a therapeutic agent although I have not conducted any studies in the area; nor have I taken part in its commercial production.

7. In or about 1992, I learned of Dr. Ascuai's and Dr. Falk's invention discussed in Application Serial No. WO 91/04058 which entered the National Phase, I am advised, in the United States as Application Serial No. 07/675,908 (this Application) and subsequent applications PCT/CA 93/00061 published under International Publication No. WO 93/16732 and Application PCT/CA 93/00062 published International Publication No. WO 93/16733 which latter two publications related to the topical treatment of basal cell carcinoma, actinic keratosis, the topical treatment of pain and other conditions and diseases for which I understand corresponding U.S. Patent Applications have been filed.


8. To put my reaction to the invention of WO 91/04058 in basic terms, the use of hyaluronic acid for transporting (delivering) drugs and therapeutic agents



to the locations of diseases and conditions, appeared to me at the time and appears to me even today, to be a novel and very exciting discovery.

9. When I learned about the invention, and particularly, with respect to the intravenous delivery of drugs to malignant tumours using hyaluronic acid, I was at first very sceptical for one reason: beginning in 1973, I had shown in an extensive series of investigations in several species of animals and in humans that HA was very quickly eliminated from the blood stream, largely by absorption and destruction in the liver. Between about 15% and 70% of the blood's normal content of HA is removed every minute. Any drug carried by HA, I expected, would therefore be released at the lining cells in the blood vessels of the liver where HA is taken up and destroyed and the drug would subsequently be disseminated through the body via the blood stream, so I thought, as if it had been injected intravenously without HA. At the most, it might briefly create a high concentration in the liver which is so often the site of secondary cancer spread. This, I thought, could be a beneficial local effect but would have no particular advantage in treating any primary or other secondary tumour deposits should they occur elsewhere.

10. After, however, hearing Dr. Rudy Falk give details of the mode of administration used in intravenous therapy with HA, I changed my views. I had not previously known the amounts of HA administered and my reservations were based on the assumption that they were in the same order as the amounts that entered the blood stream in normal body function. They were in fact, I now discovered, very much larger and from the data that my colleagues and I had previously established, I calculated that the amounts were sufficient to sustain a high level of the administered HA in the blood stream for many hours. This would allow time for HA to permeate through the small blood vessels (capillaries) into the tissues, a phenomenon we had already demonstrated in




animals, and to carry with it any associated drug. Since the blood vessels in malignant tumours are often much more permeable than normal vessels, I concluded that the major reasons for my doubts had been resolved. Subsequent research has reinforced my conclusion.

11. I also heard the same from Dr. Asculai, who had called on me in about 1992 in Melbourne, I believe on the recommendation of Professor Torvard Laurent of Uppsala University. In a meeting with Professor Ross Cahill of the University of Melbourne and me, Dr. Asculai presented details of the results achieved in the treatment of basal cell carcinoma of the skin (ECC) with diclofenac applied in a hyaluronan gel as discussed in the other two applications referred to in paragraph 7 above (WO 93/16732 and WO 93/16733). He also discussed the outline of the use of hyaluronan (HA) to deliver drugs in the systemic treatment of advanced cancer of various kinds taught in WO 91/04058 which was discussed above.

12. For the purposes of this Declaration, I was asked to review International Application No. PCT/CA 90/00306 published under International Publication No. WO 91/04058 with a view to determining what the said document teaches to persons skilled in the art.

13. In this regard, I was asked to determine what International Publication No. WO 91/04058 specifically teaches and if the said teachings could be used and understood by persons skilled in the art having regard to the teachings therein so that the teachings could be easily and readily used by persons skilled in the art to treat patients.

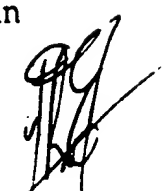
14. Having read the said document, International Publication No. WO 91/04058, I have concluded that the inventors, Drs. Falk and Asculai, have



discovered and disclosed the use of dosage amounts comprising effective dosage amounts of medicines and/or therapeutic agents together with specified amounts of forms of hyaluronic acid for transporting (delivering) the medicines and/or therapeutic agents to under-perfused tissue and/or pathological tissue. The specified amounts of the form of hyaluronic acid in the dosages alters the distribution and performance of the medicines and therapeutic agents in the body and produces an unusual targeting for the under-perfused tissue and/or pathological tissue (see p. 24, lines 13-17) of International Publication No. WO 91/04058.

15. Thus, the invention does not relate to the mere combining of known medicines and therapeutic agents with hyaluronic acid; the invention of International Publication No. WO 91/04058 relates to the use of the form of hyaluronic acid for targeting the medicines/therapeutic agents for better performance. By using the forms of hyaluronic acid with medicines and their therapeutic agents that would be known (or may become known in the future) to persons skilled in the art for the treatment of a disease or condition then, according to the inventors, if the said medicines or therapeutic agents are useful for the treatment of said disease or condition, the medicine/therapeutic agent is effectively transported to the site in the body where the treatment is intended to target the medicine or therapeutic agent, as the case may be, to the site of the disease/condition in need of treatment.

16. Would persons reading said International Publication No. WO 91/04058 have sufficient information and teaching in the document to enable them to so use the invention? In my professional opinion, they would, having regard to the teachings in International Publication No. WO 91/04058. The dosage amounts can be administered in the usual method for example, intravenously, intra-arterially, intraperitoneally, intrapleurally, transdermally, on the skin



(topically) rectally, orally or by direct injection for example, into a tumour, abscess, or similar disease focus, or put on a patch to be secured to the skin of the patient (p. 18, lines, 2-7). Persons skilled in the art would understand the routes of administration so specified and would administer the said dosages by other methods as they would know.

17. In my opinion, persons skilled in the art would be highly skilled and would have a full understanding of drugs and medicines used in, for example, the disease or condition being treated such as cancer and, therefore, would be capable of interpreting the pharmacological data in International Publication No. WO 91/04058.

18. There might well be aspects that are not immediately understood or recalled by such persons skilled in the art. However in such circumstances such persons skilled in the art with the qualifications specified would have the professional capacity and would also have the basic scientific education necessary to seek the required information and understanding from standard known textbooks and other known sources. They would also know the terms used in the Application for example, such as, "calcium channel blockers" which includes nifedipine which is mentioned at p. 35, line 23 with reference to sub-paragraph 16 of the said International Publication No. WO 91/04058.

19. Such persons skilled in the art would also understand that the varying doses of 10 mg. to 1,000 mg. of the form of hyaluronic acid per 70 kg. person are those which work with the optimal doses tending to range between 50 and 350 mg. of hyaluronic acid per 70 kg. person (see p. 26, lines 33-34).

20. There is mention at p. 26, line 35 of the statement:




As there is no toxicity, the hyaluronic acid can obviously be administered in a dose excess (for example, 3,000 mg./70 kg. individual) without any adverse effects."

I have considered this statement and this statement is generally true.

21. I am aware of a condition known as hyperviscosity syndrome which is caused by excessive amounts of abnormal blood proteins related to immunoglobulins. These can raise the viscosity of blood and increase the work load of the heart, leading to impaired circulation and in some instances, provoking heart failure. This is a rare condition and I only point this out because I have been asked to bring out all comments with respect to the Application and its statements. This rare condition most likely occurs in the elderly and those with accompanying unrelated heart disease. It is caused by malignancy such as multiple myeloma and would, therefore, be known to experts in cancer treatment. Hyaluronic acid administered directly into the blood stream could theoretically cause a similar condition owing to its effect on blood viscosity. The viscous effect of hyaluronic acid depends on its molecular weight and its concentration. (For illustration and for further references see Bothner H. Wik O., "Rheology of intraocular solutions" in Viscoelastic Materials, Basic Science and Clinical Applications, Ed S. Rosen, Pergamon Press, New York, 1989, pp. 3-22.)


22. The final concentration of hyaluronic acid in blood plasma will depend on the amount injected and plasma volume of the recipient. Thus, a dose of 3,000 mg. could raise the plasma level to about 0.25 mg./ml. after equilibrium is reached. This fact, taken with the molecular weights given for two sources of hyaluronic acid in International Publication No. WO 91/04058 (150,000-225,000 daltons and less than 70,000 daltons) which are relatively low compared with



those injected in joints, would not, in my opinion, lead to any danger of hyperviscosity heart failure.

23. Hyperviscosity heart failure caused by elevation of plasma hyaluronic acid resulting from disease seems to be exceedingly rare in the medical literature. In one example, due to Wilms' tumour, the plasma levels were very much higher than that referred to in paragraph 22 above. It should also be noted that the molecular weight of hyaluronic acid is reduced during circulation in the blood stream. In this regard, I attach as **Exhibit 2** to this my Declaration, a copy of an article entitled "*Changes in the relative molecular mass of circulating hyaluronan*", written by me, Second International Workshop of Hyaluronan in Drug Delivery, published in 1995, p. 32 (see publication #124 of my bibliography). I, therefore, conclude that, while it is possible that larger amounts of hyaluronic acid than those quoted in International Publication No. WO 91/04058 might theoretically cause hyperviscosity heart failure, it is very unlikely, in my opinion, that this would happen so that generally, except for the rare condition referred to, the dosages referred to at page 26 of International Publication No. WO 91/04058 can be or exceed 1.000 mg./70 kg. person.

24. This caution does not apply (and has no meaning or adverse effect) to administration by other routes (surface of skin, injection into tumours, pleural activity, peritoneum, etc.) where hyaluronic acid is removed either by metabolic degradation in the tissues or through the lymphatic system which absorbs most of it and also reduces the size of any polymers that pass on to the blood stream (see Fraser, Cahill, Kimpton, and Laurent, "*The Lymphatic System*" in *Extracellular Matrix*, Vol. 1: Tissue Function, Chapter 5, pp. 110-131., 1996 Ed. W.D. Comper, Harwood Academic Publishers). If the source of hyaluronic acid is sufficiently purified to be free of any unrelated toxic material, the foregoing is the only adverse effect that I can foresee arising from the intravenous injection of



hyaluronic acid in amounts larger than those specified at page 26, lines 32-37 of International Publication No. WO 91/04058.

25. With respect to molecular weights used of hyaluronic acid, I refer to p. 29, lines 34-35 of International Publication No. WO 91/04058 which discloses the use of a 15 ml. vial of sodium hyaluronate, 20 mg./ml. being a 2% solution, the solution being prepared to present hyaluronic acid in sterile water with a mean average molecular weight of about 225, 000 daltons.

26. The hyaluronic acid used referred to at page 30 of the Application may have a molecular weight range of 150,000 to 225,000 daltons having a specified pH. At page 31, another amount of hyaluronic acid is specified at line 33-34 as a viscosity average molecular weight less than 750,000 daltons.

27. International Publication No. WO 91/04058, therefore, specifies molecular weight of hyaluronic acid having a range between 150,000 to 750,000 daltons in the exemplified samples of hyaluronic acid.

28. Having regard to the teachings with respect to molecular weight, I have determined from the teachings of International Publication No. WO 91/04058 that persons skilled in the art would experience no difficulty in preparing the dosages useful for treating the disease or condition which they are desirous to treat. The hyaluronic acid dosage amounts prepared from the hyaluronic acid exemplified in the teachings of the patent would, in my opinion, be diluted by persons skilled in the art for several reasons before use in administering to the patients. The dilution of the hyaluronic acid would be clearly understood by persons skilled in the art. The first reason for diluting is for forming the mixture with the drug. The second reason would be to avoid high immediate concentrations of drug in the blood stream which also governs the rate of

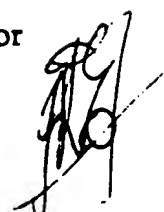


infusion. Thirdly, dilution would be carried out by persons skilled in the art to ensure smooth flow into the venous line where the dosage was to be administered intravenously. The volume and rate of intravenous infusion would be decided to accord with standard practice for intravenous infusions.

29. I have also been asked to comment upon the nature of the salts of hyaluronic acid that are to be used. The necessity for use of non-toxic salts of hyaluronic acid when implementing the teachings of International Publication No. WO 91/04058 is clear to me, and would be clearly to any person skilled in the art, self-evident. No physician would use, for example, a toxic amount of a salt such as lithium salt which is potentially toxic. Persons skilled in the art would only use non-toxic salts or pharmaceutically acceptable salts. The expression "salts, non-toxic salts, and pharmaceutically acceptable salts" are within the understanding of persons skilled in the art to be interchangeable when referring to the salts in International Publication No. WO 91/04058.

30. Based on the above, it should be clear to the reader that persons skilled in the art, from the teachings in the Application, will, in preparing the dosage form, select a suitable form of hyaluronic acid from the criteria discussed above and combine the appropriate amount of hyaluronic acid with the effective amount of the medicine/therapeutic agent which such person would know to be suitable or to be useful in the treatment of the disease or condition to be treated. Such person would prepare the combination in suitable dosage amounts without much experimentation, if any, having regard to the many examples given in the Application. Such person would then administer the dosage amount.

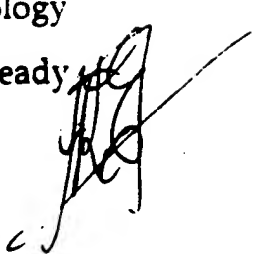
31. The objects of using the hyaluronic acid in conjunction with the medicines/drugs chosen for the specific purposes for treating the conditions/diseases which are either specifically described in the Application or



clearly taught in the Application as would be understood by persons skilled in the art, would thus provide enhancement of therapeutic efficacy of the drug by selective distribution of the drug to the desired site of action and the achievement of more effective and sustained concentrations of the drug at the site, in effect, to achieve a better result with fewer side-effects.

32. I was also asked to provide my opinion with respect to spontaneous remission and its effects on the teachings of this invention and, in my professional opinion, having regard to the teachings herein, spontaneous remission is not likely to be relevant. Firstly, to my knowledge, spontaneous long-term remission of proven cancer appears to be an exceedingly rare event. The patients having cancer whose treatment regimen was exemplified beginning at page 36 have been unresponsive to conventional treatment (see p. 36. lines 2-6) means to me the patients were terminally ill (they were going to die from the cancer). With basal cell carcinoma, spontaneous remission is unlikely; in my opinion, the successful treatment in International Publication No. WO 93/16732 and WO 93/16733 arose, not from spontaneous remission but, from the formulations taught therein, and the dosage amounts administered over the period of treatment. I was particularly impressed by the treatment of one patient with the inventions in International Publication No.'s WO 93/16732 and WO 93/16733 who permitted, by self-neglect, the basal cell carcinoma to progress to a very advanced state, which state was regressed by the treatments as described. This regression could not, in my opinion, be attributed to spontaneous regression. The cause of the regression was the application of the HA and Diclofenac gel.

33. The details given in International Publication No. WO 91/04058 are sufficiently clear to enable a qualified practitioner to adopt the methodology without undue experimentation and achieve the results therein. I have already

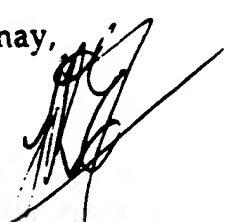


seen one colleague do so in a patient with advanced cancer with ethical and official Pharmaceutical Advisory Committee approval. The responsible oncology team had no difficulty in formulating the procedure and observed no untoward effects.

34. To me, the case particulars following page 36 of International Publication No. WO 91/04058 teach exemplary dosages and methods of treatment using the dosages. Even patients who did die appeared to have had some positive response to the dosages administered. Many of the terminally ill patients did survive much longer than one would expect from the clinical data given.

35. Persons skilled in the art would thus, in my professional opinion, adhere to the examples given for the purposes set out and would use molecular weights of hyaluronic acid of between 150,000 daltons to 750,000 daltons and would dilute same for administration with the medicines as taught in International Publication No. WO 91/04058. No undue experimentation would be required. International Publication No. WO 91/04058, in my opinion, teaches persons skilled in the art, all that is needed to implement the invention.

36. I was aware of the article by West *et al.*, 1989, and other articles by these authors, before being asked to comment on their teachings, by Ivor Hughes, Counsel for Hyal Pharmaceutical Corporation. West was, according to Mr. Hughes, purportedly read by the U.S. Examiner to state that *in vivo* studies indicate that a hyaluronate-rich stroma inhibits blood vessel formation in chick limb buds whereas low molecular weight oligosaccharides of HA stimulate angiogenesis *in vivo* and endothelial cell proliferation *in vitro*. West *et al.* is purportedly suggesting that this finding is an important consideration for tumour growth since tumour growth depends on angiogenesis. Thus, according to West *et al.*, it is asserted by the Examiner that low molecular weight HA may,

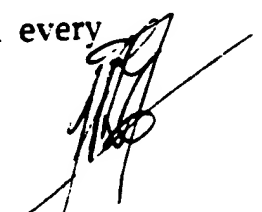


in fact, promote the growth of tumours and therefore, low molecular weights would not have the properties that make it useful for the treatment of disease and penetration according to International Publication No. WO 91/04053.

37. The studies of West and his colleagues are very well known and understood by persons skilled in the art. West et al. reported that only HA derived oligosaccharides in the range of 3-10 and 10-16 disaccharide units which stimulated the proliferation of bovine aortic endothelial cells. These HA derived oligosaccharides would cover a molecular weight range of 1,200 to 6,400 daltons. This range is very much lower than those given in International Publication No. WO 91/04058. It cannot be assumed that the molecular weights of HA in International Publication No. WO 91/04058 would have similar effects. For example, the individual molecules would not form the stiffened random coil configuration of the big polymers which are capable of entraining large amounts of small drug molecules.

38. Furthermore, West's conclusions that HA oligosaccharides do, in fact, promote growth of tumours through angiogenesis is far from proven. As West and Kumar say in their discussion, endothelial cells from different sources within the same species (bovine) may respond differently. While their experimental findings are interesting they cannot be extrapolated to events within the living body. As I also understand, Toole et al. cannot duplicate the results. Paul Noble found that lower size fragments stimulate all things that West has found. If the Examiner has cited West for the purposes for which I am advised, as discussed above, in my professional opinion, the Examiner is mistaken.

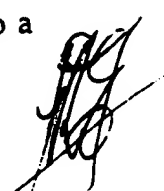
39. Knowledge of effective and non-toxic amounts of medicines and therapeutic agents is the primary responsibility of the professional in every



instance. International Publication No. WO 91/04058 clearly indicates that delivery of a therapeutic agent with hyaluronic acid or its salts may achieve an enhanced therapeutic response. The forms of treatment canvassed in the Application (exemplified in the Application) do not entail the withholding of orthodox treatment but, advocate, in my opinion, their reinforcement by administration with hyaluronic acid, a natural component of body tissues which could be reasonably assumed to be well tolerated. The approach taken by Drs. Falk and Asculai avoids the ethical problems of simply comparing a therapeutic agent of unpredictable efficacy with established methods of known value. The initial trials in patients with terminal cancer without controls rest heavily on clinical evaluation which, in turn, relies on historical experience of the evaluator. The outcome appears to have been sufficiently impressive ~~to~~^{for} me to conclude from these results that hyaluronic acid enhances the effect of the therapeutic agents in patients likely otherwise to be resistant to standard therapy (see page 36, lines 2-6 of International Publication No. WO 91/04058). Thereafter, further evaluation would proceed through a well established sequence ending with carefully designed control trials. However, it is clear to me when dealing with patients having cancer and who are terminally ill, the historical controls are appropriate. If the patient has been found to be terminally ill, the professionals now conclude that the patient will die unless another treatment is successful. Spontaneous remission is remote and not expected.

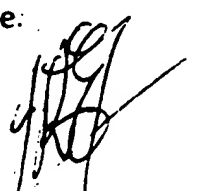
40. In view thereof, it is in my professional opinion that it is not an impropriety for International Publication No. WO 91/04058 not to contain any controls as persons skilled in the art would appreciate that when a patient has been found to be unresponsive to conventional treatment, the patient will die.

41. One last point deserves mentioning. Drs. Falk and Asculai also discovered that when 200 mg. of hyaluronic acid is given with an NSAID in the dosage to a



person being treated with an NSAID (see for example, page 25, lines 20-21 and 34-35), no major toxic side effects occur, which would be expected from administering the NSAID alone, such as gastrointestinal distress, neurological abnormalities, depression, etc., even when the NSAID is administered at elevated amounts. The gastrointestinal and neurological side effects of NSAIDS are well known when administered alone.

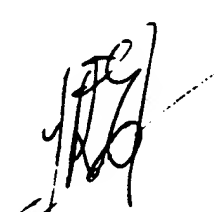
The explanation for such preventive effect is, however, a different matter. When delivering either standard or excess dosage amounts of the medicine, a professional would, in my opinion, immediately refer to standard sources of information if in doubt, and would have such sources close at hand. Such professional would also have the examples in the patent and where, for example, doses containing in excess of 300 mg. of an NSAID are given to a patient and, such patient was still bothered by the NSAID, because the hyaluronic acid, while eliminating some of the side effects, did not eliminate all side effects of the NSAID because of the unusually high amount of NSAID of 300 mg. (see for example, p. 53, line 35), the dosage of the NSAID may be reduced to, for example, a lesser amount such as 100 mg. (still an excess dosage amount). In Example XIX of International Publication No. WO 91/04058, the NSAID caused heartburn in the patient but, when the NSAID was reduced to 100 mg., which is still considered to be an excess dosage amount, the patient could tolerate same. This excess dosage amount of 100 mg. of NSAID indomethacin was still tolerated by the patient and the side effects did not materialize and interfere with the patient's taking of the dosage amount. This patient was not comfortable taking 300 mg. of NSAID. International Publication No. WO 91/04058, however, discloses other patients treated with dosages of 300 mg. of indomethacin with hyaluronic acid (see for example, Case VIII, p. 45, line 25 and Example XVIII at page 53, line 2) and there do not appear to have been objections to this treatment by the patient. The patient appears to have been able to tolerate same.



Thus, the amount of NSAID useful to treat the patient with the hyaluronic acid would depend on the patient's sensitivity to side effects. In any event, International Publication No. WO 91/04058 teaches the reduction of side effects of the NSAID using hyaluronic acid and there is no reason to doubt the teachings.

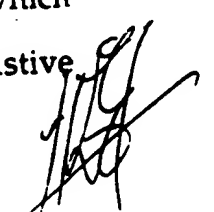
42. I have been asked to review the teachings of Seifter U.K. Patent 769, 287 specifically as to what Seifter does and does not teach. I have wrestled with the teachings of Seifter. In wrestling with the problem, I asked Counsel for Hyal Pharmaceutical Corporation, owner of the Application for which my professional opinion was sought, to conduct a publication search to determine if Seifter may have published his teachings in a publication wherein the teachings in Seifter, U.K. Patent 769,287 have been amplified to make them more understandable and enable me to determine what, in fact, has been happening. I was advised by Ivor Hughes that no publication was found in his search.

43. A memorandum was submitted to the Patent Office, a copy of which I attach as *Exhibit 3* to this my Declaration, for which I assisted in the preparation. This memorandum has already marshaled excellent evidence that the initial molecular weight of Seifter's HA could have varied enormously since Seifter et al. quotes sources other than streptococcal product. Seifter's conclusions are that hyaluronic acid, which has not been depolymerized, had practically no useful effect but, that partially depolymerized hyaluronic acid had a spreading effect. Thus, if the molecular weights of the undepolymerized hyaluronic acid and polymerized hyaluronic acid were for example, the same (and assuming this is possible), one would work (the PDHA) and the other would not work (undepolymerized hyaluronic acid) according to Seifter.



44. Seifter's oligosaccharides purportedly disperse the meshwork of the naturally large HA polymers in normal skin structure like hyaluronidase but without the disadvantages. The essence of Seifter's claim is that his PDHA is presented as accelerating the spread more rapidly through skin in a manner akin to the effect of hyaluronidase which had already been established. The breakdown of the molecular meshwork of the naturally large HA polymers, which form a major part of the skin, reduces the resistance to movement of other substances through the matrix in the skin, both by diffusion and particularly, by the flow of water through the tissue from blood to lymph. The spreading effect would dilute the agent (drug) accompanying the PDHA, accelerate dispersal of the drug into the blood stream and would remove it more rapidly from any desired target in the skin or regional lymph nodes which would be the antithesis of the objective accomplished in International Publication No. WO 91/04058. These activities will reduce both the effective concentration of the therapeutic agent and its period of persistence in the skin.

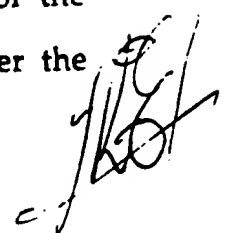
45. Regardless of the mechanisms by which PDHA produces these effects, it should be apparent that they are diametrically opposite to the objectives of International Publication No. WO 91/04058 which are to deliver a drug to a specific area affected by a disease process. The Examiner has previously suggested a range of molecular weights that were suggested to be those of Drs. Falk and Asculai's molecular weights for hyaluronic acid (undepolymerized). In that event, such PDHA molecules are large enough to be incorporated in the existing HA meshwork in the skin matrix and would, therefore, be unlikely to promote spreading of their substances. However, PDHA, in my opinion, is HA-derived oligosaccharides between six (6) and twelve (12) single sugars which break up the bonds that HA forms with various other large molecules and are most likely break up the meshwork of the naturally large hyaluronic acid polymers which form a major part of the matrix of the skin. The end-product of exhaustive



digestion of HA with hyaluronidase is tetrasaccharide, a four (4)-sugar residue. This is too small to disrupt the bonding of an HA molecule to other molecules but, before this point is reached, there will first be an increasing proportion of oligosaccharides in the above range and then a decrease as the digestion approaches completion. This would certainly be consistent with the time course recorded in the generation of spreading activity by the enzymatic treatment of the initial HA material (see page 2, lines 1-16 of Seifter, U.K. Patent 769,287). It is, therefore, clear to me, that PDHA is not the polymer, hyaluronic acid. Seifter probably did not know precisely how far his degradation had gone. If the enzyme he used was testicular hyaluronidase, it would in the earlier stages generate an increasing proportion of the larger oligosaccharides, which would then be reduced in amount as the enzymatic digestion approached completion. It has been shown in recent years that hyaluronan-derived oligosaccharides can disperse the gels of hyaluronan generated around cultured tissue cells, which is presumed due to their loosening bonds that hold the polymers together. Such an effect would also promote the spreading of small molecules (e.g., water, salts, dyes, and drugs) through the natural hyaluronan network in the tissues of skin.

46. I feel compelled to conclude, in any case, that it is impossible now to compare Seifter's PDHA to any hyaluronan polymers with their molecular weight specified by the methods available at the time or available now.

47. Regardless of the mechanisms by which PDHA produces these effects, it should be apparent that the effects are diametrically opposite to the targeting and delivery of Drs. Falk and Asculai in International Publication No. WO 91/04058; their objective is to target a site of disease or condition providing an unusual targeting for under-perfused tissue and/or pathological tissue. In other words, there is no dissolution in International Publication No. WO 91/04058 of the therapeutic agent caused by the spreading effect into the skin but, rather the

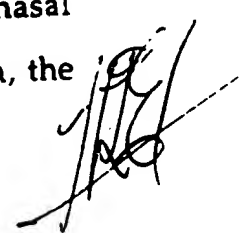


specific targeting to the tumours. The PDHA, whatever it is, acts differently and causes dilution. The PDHA of Seifter is, in my opinion, the much smaller oligomer and not the polymer HA.

48. I have also been asked to examine Della Valle et al., U.S. Patent 4,736,024, Schultz, U.S. Patent 4,808,576, and Balazs et al., Hyaluronic Acid: Its Structure and Use, Polymers in Cosmetics, 1984, Vol. 99, pp. 65-72 and provide my comments with respect to the teachings of each.

49. Della Valle, U.S. Patent 4,736,024 exemplifies what was known in the industry in about 1987 in respect of topically applied formulations containing HA and substances carried by the HA when applied to the eye for absorption of the substance carried in the HA by the eye. The data given makes it clear to me that the said formulations, if applied topically, cause the HA to adhere to the eye (the cornea is specified) and permit the substance carried by the HA to leach or leak out therefrom and be absorbed by the underlying tissue. This is to what precisely the teachings of Della Valle, U.S. Patent 4,736,024 relate.

50. Della Valle provides combinations of HA and medicine but, does not provide appropriate dosages to achieve the delivery and transport of the medicine as is taught by International Publication No. WO 91/04058. While Della Valle discusses the use of the formulations for ophthalmics (the eye) and suggests the use in dermatology, the patent also suggests their use, at column 4, that it is possible to conclude by analogy that the medicines can be used in other fields such as otorhinolaryngology, odontology, or in internal medicine for example, endocrinology where it is possible to effect treatments with preparations for intradermic absorption or absorption through the mucous membranes for example, rectal or intranasal absorption, for example, nasal sprays, or inhalations in the oral cavity and in the pharynx. In my opinion, the



nature of the formulations used elsewhere than the eye does not change. It is expected that the formulations will, once again, stick to the surface to which they have been applied and from which the medicines will leach. This retard effect of the HA and leaching effect of the medicine from the HA are only substantiated, however, for the special case of the cornea and only by analogy does Della Valle suggest the conclusions may be applied to topical formulations applied elsewhere topically.


51. I have arrived at this conclusion based on the teachings of the patent as a whole and refer the Examiner's attention to column 1, line 46:

"When the medicaments are administered in the form of concentrated solutions with the elastic-viscose characteristics or in solid form, it is possible to obtain films on the corneal epithelium which are homogenous, stable, perfectly transparent and which adhere well guaranteeing prolonged bioavailability of the drug thereby forming excellent preparations with a retard effect."

52. A similar statement is made at column 2, lines 41-51.

It is therefore clear to me and would be to persons skilled in the art that the better bioavailability of the active substance, which results are achieved by the use of the hyaluronic acid, provides a film on the surface of the eye which adheres well and which forms a preparation with a retard effect.

53. Such a formulation is contemplated for use with the fields discussed at the bottom of column 4, top of column 5, namely, the topical application and the leaching therefrom.



54. This conclusion is clearly reinforced by the dosage amounts given in the examples in the patent. At column 27, line 57, the formulations referred to in the example are administered by a microsyringe (10 mcl). The largest concentration of hyaluronate is provided at a concentration of 20 mg./ml. (column 27, line 49). On that basis, a dosage from a microsyringe (10 mcl) contains ^{0.2}~~0.2~~ mg. of hyaluronic acid.

55. The same use is set out at column 29, line 30 wherein the animals were infused with a microsyringe (10 µl). The reader is also directed to column 30, line 37 where one drop, 50 µl is instilled into the eye. At the bottom of column 30, the statement at line 65 appears:

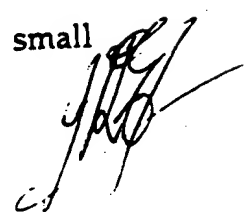
"Transcorneal penetration of pilocarpine seems therefore to depend on the capacity of hyaluronic acid to vehicle the drug forming a homogenous and stable film on the cornea."

There is no transport by hyaluronic acid beyond the surface of the eye. The transcorneal penetration is by the pilocarpine itself.

56. Other drops are discussed at column 31, line 52-53 and column 33, lines 23-24.

57. It is therefore clear to me that while combinations of hyaluronic acid with medicine have been taught to a limited extent, the dosage amounts taken from these formulations are minuscule and are for purposes and effects which do not provide HA for transportation and delivery of the medicine within the tissues.

58. As can thus, now be seen, the combination of Seifter and Della Valle teaches nothing. Seifter does not use hyaluronic acid. Della Valle teaches small

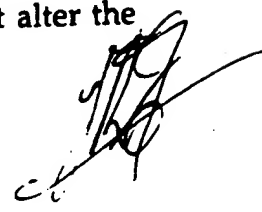


dosage amounts which cannot transport (deliver) the medicine to site in need of treatment.

59. Test data is provided by Della Valle only in respect of ophthalmic applications by demonstrating that adherent and stable films form on the cornea of the eye. The formation of such a film and the viscosity of HA itself would separately and/or together reduce the effect of tear secretion in washing away the drug and thus, provide an obvious explanation for the effect of HA on ophthalmic medications thus, purportedly providing a retard leaching effect. However, no data is provided to support claims for similar results in application to other sites. There is no indication that such usage has been studied and no examples of suitable preparations or evidence of their use at these sites are given. There is no reference to the use of the amounts of HA taught in Drs. Falk and Asculai's Application under consideration herein including no discussion of the use of greater than 200 mg. of the hyaluronic acid or to the reduction of side effects of the taking of the medication.

60. Thus, there is nothing in the U.S. Patent 4,736,024 to teach even the remote possibility that HA can act as transport agent for an associated drug. The specification that the drug should be capable of absorption through the surface of the skin or mucous membranes of the nose, throat, or rectum clearly implies that, even in its transport ^{for this} ~~of the~~ short distance, the drug must be transferred into the skin, ^{and} the drug must rely on the intrinsic properties of the drug itself rather than carriage by hyaluronic acid.

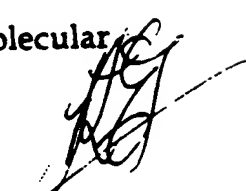
61. The fact that Della Valle reacts HA with drugs (by the reaction of the acidic [carboxyl] groups and drugs that are basic) which might favour a retard effect of the release of the drug if they dissociate slowly after application does not alter the teachings of Della Valle.



62. In summary, therefore, the patent does not teach the positive transport or delivery of the medicine by the hyaluronic acid to the site in need of treatment. Rather, the HA only maintains the medicine in a film topically where the therapeutic agent must have the specific specifications capable of being absorbed which clearly requires that the HA is not considered by Della Valle to be an agent for transport of the drugs within or through the tissues.

63. Clear reference is made to a retard effect which is based on the formation of a homogenous stable adherent film on the cornea (column 1, lines 48-53). This is attributed to HA providing a more efficient vehicle and better bioavailability (column 1, lines 35 et seq.). These observations made on the behaviour of the preparation in the eye constitute evidence for the last statement (providing a more efficient vehicle and better bioavailability) rather evidence of it. This is an important distinction since the nature of the cornea is quite distinct from that of the surface of the skin or mucous membranes and its interactions with HA cannot be held applicable to the latter as a matter of course as stated at column 2, lines 53-55. It is, therefore, not clearly apparent that the teachings of the formulations with the eye will apply to odontology, dermatology, etc. as specified by Della Valle. In any event, the formulations would be expected to have the same effect as taught with respect to the eye, the preparation of a film from which the medicine leaches.

64. Schultz U.S. Patent 4,808,576 refers to the use of HA itself as a therapeutic agent. It specifies a molecular weight at least 500,000 and preferably in a range between 1.2 and 4.0 million. High viscosity is recommended for topical application, and low viscosity for other routes of administration, the viscosity being measured as a "1 weight % aqueous solution". If the concentration is fixed in this way, a difference in viscosity implies a difference in average molecular

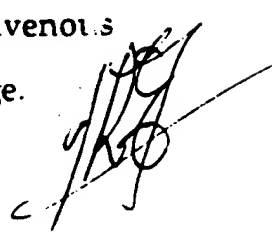


weight, even if the measurements are made at a high rate of shear, which would be likely to pertain in a capillary viscometer. This section of the specifications is far from clear.

65. Throughout this document, it is made clear that HA itself is the therapeutic agent. It is stated that topical application of HA requires a "compatible" and "recognized" "transdermal carrier" and cites methyl or sodium salicylate, benzyl alcohol, oleic acid (amounts of these agents are not specified) and other, particularly DMSO. Although the salicylates and DMSO are themselves therapeutic agents, they are referred to here only as transdermal carriers for HA. In the Schultz Example 1, HA was injected into muscle without carrier. The transdermal carriers are used only to transport HA through intact skin. Thereafter, the invention relies on "the fact that the internal transport systems of the mammalian body are effective in conveying hyaluronic acid to the affected site". It is not stated whether this occurs in the course of a general dissemination of HA given by the various routes that would invariably encompass any injured tissue.

The great variety of drugs quoted by Della Valle and associates would not be seen by the reader as potential transdermal carriers of hyaluronan. I cannot find anything in the Schultz patent to indicate that HA might facilitate the penetration of the transdermal carriers. This would be a distinct concept.

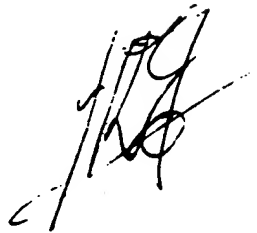
66. The claims specify reduction of inflammation, pain or other result of trauma in irritated mammalian tissue including adhesions in surgery, by an "effective" dose of hyaluronic acid or an acceptable salt thereof. The specified dose of HA can be administered topically, subcutaneously, intramuscularly or intravenously. Other details are given but no example of intravenous administration is given and there is no specification of intravenous dosage.



67. The Schultz patent is not relevant to procedures that use HA as the vehicle for another therapeutic agent or as a means to deliver other therapeutic substances to a desired site of action.

68. When attempting to read Della Valle, U.S. Patent 4,736,024 with the teachings of Schultz, U.S. Patent 4,808,576, the great variety of drugs quoted by Della Valle would not be seen by the reader as potential transdermal carriers of hyaluronan. Nor is there anything in Schultz to indicate that HA might facilitate the penetration of the transdermal carriers. This patent, therefore, in my opinion, could not be combined with U.S. Patent 4,736,024 to arrive at Applicant's invention. This patent is not relevant to procedures that use HA as a vehicle for another therapeutic agent or as means to deliver other therapeutic substances to a desired site of action beyond the immediate vicinity of the site of application.

69. Combining Schultz, Seifter and Della Valle does not yield the invention in International Published Application No. WO 91/04058. Even reading them and looking for a motivation to combine them, there is none. There is no reason or motivation to pick and choose bits and pieces of each of the references because not one of them teaches the use of HA itself as an agent to carry and deliver a drug or medicament beyond the point of application to a remote site of disease where concentration of the treatment will be most effective and possibly, with fewer side effects. This delivery taught by International Publication No. WO 91/04058 is totally unexpected. The documents do not, in my opinion, share any fundamental identity with the technology developed by Dr. Falk and Asculai and taught in International Publication No. WO 91/04058.

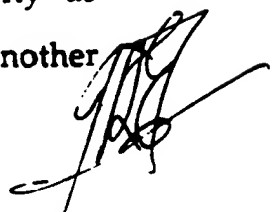


70. The article entitled "Hyaluronic Acid: Its Structure and Use, Polymers in Cosmetics, André A. Balazs, M.D., et al. adds nothing to the other three references and discusses the water retaining abilities of hyaluronic acid with respect to human skin. He notes its presence in the dermis and comments that it is the predominant GAG produced by human and animal epidermis in culture. He observes that high molecular weight hyaluronic acid solutions (I expect high molecular weight to be in excess of 1 million to 2 million daltons) when applied to the surface of skin form hydrated viscoelastic films. These films hold water in the same way that hyaluronic acid holds water in the intracellular matrix of dermal connective tissue. The hyaluronic acid thus forms an underlying film on the skin which is completely transparent without a tacky or greasy feel and is perceived only by its softening, smoothing, and lubricating influences. The article states on the last page, before the references, second-last paragraph as follows:

"The stratum corneum ~~is~~ is known to be impermeable to molecules as large as hyaluronic acid, therefore, it is not expected that even very short chains (oligosaccharides) of degraded hyaluronic acid that contain more than five to ten disaccharide units can pass through this layer of the skin. There is no evidence in the literature that any hyaluronic acid - in any solvent, or with any added carrier - will penetrate deeper than the crevices between the desquamating cells."

This document does not teach or even suggest the transport (delivery) of an agent to a site or a condition or disease in need of treatment.

71. When Dr. Asculai contacted me in Melbourne, in 1992, I believe on the recommendation of Professor Torvard Laurent of Uppsala University as previously discussed, he presented the results achieved in respect of another



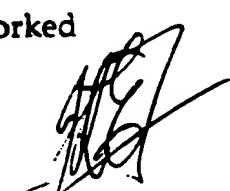
invention used for the treatment of basal cell carcinoma of the skin (BCC) with diclofenac (see International Publication No.'s WO 93/16732 and WO 93/16733) applied with the hyaluronan gel and an outline of the use of hyaluronan (HA) to deliver drugs in the systemic treatment of advanced cancer of various kinds. I had been involved with hyaluronic acid since the '70's and I had never heard of anything like this. There was nothing in the literature.

72. With respect to the topical treatments, I was immediately impressed by the former, especially by the regression in the case of BCC which was very advanced due to self-neglect on the part of the patient. It might seem strange to many physicians and scientists that diclofenac, a simple non-steroidal anti-inflammatory drug, not expected to have a direct anti-cancer effect in ordinarily tolerated dosage (NSAID), should cause regression of a tumour. At that time, it was not widely known that the tumour cells in BCC were surrounded by a matrix containing hyaluronectin, which specifically binds HA, and that in common with more aggressive cancers, the matrix of BCC also contained endogenous HA. The hyaluron^{an}~~ectin~~ can act as an impedence to access by immunologically competent cells such as lymphocytes and macrophages that might attack and kill the cancer cells as being "foreign" to the body. The notion that the immune responses of the body might control or eliminate cancers is by no means new, and was seriously considered by outstanding pathologists decades ago. Some of the literature refers to this theory but does not refer to the tumour matrix as a potential inhibitor of immune attack on the tumour. Inhibition by the matrix might act at two vital points; firstly, by inhibiting contact with the immune cells that initiate an immune response and, secondly, by impeding contact with any immune cells that might have become specifically sensitised to, and capable of selectively destroying, the tumour cells. Direct contact is necessary for immune destruction of target cells by sensitised (cytotoxic) immune cells. The delay of some weeks in the regression of tumours with such treatment would be

consistent with the time required to suppress the tumour matrix and initiate an immune response.

73. Substances known as prostaglandins are produced by cells in response to a variety of stimuli, and play an important role in the cellular synthesis of HA. If present in adequate concentration, NSAID can block the cellular production of prostaglandins and therefore, reduce the synthesis of HA and its release into the matrix around the cells. They could thus promote a more effective immune attack on BCC cells. This above explanation provided one rational explanation for the effects that Dr. Asculai showed us, though it was also possible that a high local concentration of NSAID might have a direct toxic effect on the tumour cells. As far as the role of HA was concerned, its physical properties would at least provide a reservoir in the overlying skin having been accumulated there with the drug for gradual and sustained release of the drug from the skin directly to the tumour and, at the same time, delaying its dispersion throughout the surrounding skin and ensuring a high local concentration of drug. Bearing in mind that the skin over basal cell carcinoma (BCC) is often ulcerated or at least thinned, we considered it possible that some hyaluronan penetrated into the tumour carrying with it the entrained drug, though it was unlikely that the absorbed HA would be sufficient to interfere with any enhancement of immune reactions gained by blocking production of endogenous HA (from the tumour matrix).

74. I was therefore most impressed with the potential significance of the observations Dr. Asculai described to us and felt that methodology warranted systematic clinical investigation and further exploration of the possible mechanisms responsible for it. We, therefore, looked for the possible mechanisms and conducted research into that area. What was clear to me, however, was that the formulations and methods of treatment, in fact, worked

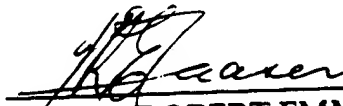


irrespective of the possible mechanisms responsible for it and were novel, useful, and not obvious to persons skilled in the art.

75. In my opinion, the subject matter disclosed in Application Serial WO 91/04058 which I understand entered the National Phase in the United States under Application Serial No. 07/675,908 is new, useful, and not obvious to persons skilled in the art.

76. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements will jeopardize the validity of the application and any patent issuing thereon.

EXECUTED this 29th day
of August, 1996.



JOSEPH ROBERT EMMOTT FRASER

EXHIBIT 1

CURRICULUM VITAE

NAME: Joseph Robert Emmott FRASER

ADDRESS: 131 Manning Road, East Malvern, Victoria, 3145.

CITIZENSHIP: Australian.

DEGREES AND QUALIFICATIONS: MB BS Melb., MD Melb.;
Fellow, Royal Australasian College of Physicians;
Fellow, Royal College of Physicians (Lond.).

ACADEMIC DISTINCTIONS: Elected to Royal Swedish Academy of Sciences, 1995
Hon. MD Uppsala, 1988;
Elected to Royal Society of Sciences, Uppsala, Sweden, 1985
Selwyn-Smith Medical Research Prize, 1972;
Reader in Medicine, University of Melbourne, 1970;
Stawell Memorial Prize (with A.J. Barnett), (Melb) 1954.

PRESENT APPOINTMENTS: The Royal Melbourne Hospital: Hon. Consultant Physician
University of Melbourne: Hon. Senior Research Associate,
Faculty of Veterinary Science.
Monash University: Hon. Associate, Department of Biochemistry
and Molecular Biology, Faculty of Medicine.

PAST APPOINTMENTS: University of Melbourne:
Department of Medicine at The Royal Melbourne Hospital:
First Assistant and Assistant Director, 1966-1978;
Deputy Chairman, 1978-1992.
Royal Melbourne Hospital:
Physician, 1966-1992.
Co-ordinator, Medical Group C, 1988-1992
(Medical Unit C, Neurology, Rheumatology);
Head, Medical Unit C, 1989-1992.
Other:
Honorary Physician to Outpatients, Prince Henry's Hospital,
Melbourne, 1960-66. (Member of Cardiology Group, a Senior
Member of Medical Department, Clinical Teacher, Melbourne &
Monash Universities.)

Lecturer in Therapeutics, University of Melbourne at Prince Henry's Hospital.

Senior Associate in Medicine, University of Melbourne.
Research Fellowship of the Arthritis and Rheumatism Council for Research in Great Britain and the Commonwealth.

Research Assistant, University of Melbourne Department of Medicine.

Resident Medical Officer (Registrar) to Drs Paul Wood, W. Evans, Evan Bedford and others, National Heart Hospital, London.

House Physician to Professor J.G. Scadding and Professor C.M. Fletcher, Postgraduate Medical School, London.

Senior House Officer, West End Hospital for Neurology, London.

Honorary Associate Physician to Dr. J.E. Clarke, Alfred Hospital.
Research Fellowship, Life Insurance Medical Research Fund of Australia and New Zealand.

Registrar, Clinical Research Unit of Baker Medical Research Institute, Alfred Hospital, Melbourne.

General Registrar, Alfred Hospital, Melbourne.

Resident Medical Officer, Alfred Hospital, Melbourne.

OTHER APPOINTMENTS AND ACTIVITIES:

Academic

Honorary Research Associate, Department of Chemical Engineering, Faculty of Engineering, Monash University, 1978-82.

External Examiner for Ph.D for various universities in Australia and New Zealand.

External referee: Chair of Microbiology in University of British Columbia; Personal Chair in University of Sydney.

Internal Examiner for M.Sc., M.Vet.Sci., Ph.D and MD theses.

Lecturer, 'Viruses and Arthritis', in Dean's Lecture Series, Faculty of Medicine, University of Melbourne, 1982.

Visiting Lecturer, Royal Newcastle Hospital, 1980.

Deputy Chairman, Board of Examiners in Medicine, University of Melbourne, 1978-79.

External Examiner in Medicine, Fiji Medical School, 1975.

Chairman, Board of Examiners Medical Studies 1, 1974-75, in the establishment of the subject in the medical curriculum.

The Queen Elizabeth Hospital Research Foundation Visiting Fellow, Adelaide, 1975.

Honorary Research Associate, Department of Chemical Engineering,
Faculty of Engineering, Monash University, 1978-82.

External Examiner for Ph.D for various universities in Australia and
New Zealand.

External referee: Chair of Microbiology in University of British
Columbia; Personal Chair in University of Sydney.

Internal Examiner for M.Sc., M. Vet. Sci., Ph.D and MD theses.

Lecturer, 'Viruses and Arthritis', in Dean's Lecture Series, Faculty of
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Visiting Lecturer, Royal Newcastle Hospital, 1980.

Deputy Chairman, Board of Examiners in Medicine, University of
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External Examiner in Medicine, Fiji Medical School, 1975.

Chairman, Board of Examiners Medical Studies 1, 1974-75, in the
establishment of the subject in the medical curriculum.

The Queen Elizabeth Hospital Research Foundation Visiting Fellow,
Adelaide, 1975.

Examiner for MD Part 1, Faculty of Medicine, University of
Melbourne, 1975-

Examiner, Australian College of Ophthalmologists' Prize, University
of Melbourne, 1970-71.

Queen Elizabeth Hospital Research Foundation Lecturer, Adelaide,
1970.

Examiner in Medicine for D.P.M., University of Melbourne,
1969-72.

Professional

Chairman, Board of Medical Research, Royal Melbourne Hospital,
1984-1992. (Member, 1978-1992).

Ethics Committee for Research, Royal Melbourne Hospital,
1984-1992.

Australian Rheumatism Association - President, Victorian Group,
1969-71.

Member, Victor Hurley Medical Research Committee, Royal
Melbourne Hospital Medical Research Advisory Committee 1978-
(Chairman of Interviewing Subcommittee, 1980-83).

Chairman, Royal Melbourne Hospital Infection Control
Subcommittee, 1980-83.

Member, Victorian State Committee, Royal Australasian College of
Physicians, 1970-71.

Scientific

President, Cell Culture Society of Victoria (now Cell Biology
Society), 1969-70.

President, Connective Tissue Society of Australia and New Zealand,
1978-80; Secretary, 1982-83.

Guest Speaker, Clinicopathological aspects of Ross River virus infection. Australian Society for Microbiology, 1981.
Guest Speaker, Australian Zoonotic Arboviruses. Australian Veterinary Association National Meeting, 1992.
Guest Speaker, Ross River virus disease. Australian Institute of Medical Scientists, Victorian State Meeting, 1992.

Public Service

Visiting Assessor, Assistance Programs for Educational Training in Universities and Department of Health, Papua New Guinea, 1981 for Australian Development Assistance Bureau, Department of Foreign Affairs, Commonwealth of Australia.

Guest Lecturer, Clinical and diagnostic aspects of virus arthritis, for

(a) Sunraysia Medical Society and Mosquito Abatement Committee, 1981;

(b) Joint Federal-States Health Surveyors Conventions, Deniliquin, 1979; Echuca, 1981.

(c) Australian National Diseases Control Program Seminar, Moama, N.S.W., 1984.

Member, N.H. & M.R.C. *ad hoc* Working Party of Arbovirus Laboratory Staff, 1984.

National Disease Control Programme - Member of Working Group on the Clinical Epidemiology of Arboviral Disease, 1986.

Member of Victorian Arbovirus Task Force, 1987-

Deputy Chairman, 1988-

MEMBERSHIP OF SOCIETIES:

Tissue Culture Association, Inc. (USA).

Biochemical Society (UK).

New York Academy of Sciences.

Connective Tissue Society of Australia and New Zealand.

Cardiac Society of Australia and New Zealand.

Australian Rheumatology Association.

INVITED OVERSEAS ADDRESSES:

Arthritis & Rheumatism Council of Great Britain.

Royal Postgraduate Medical School, London.

Kennedy Institute of Rheumatology, London.

University of British Columbia (Faculty).

University of Michigan, Ann Arbor (Faculty).

Uppsala University (Institute for Medical Chemistry).

Cornell Medical School, New York (Hospital for Special Surgery).

Mt. Sinai College of Medicine, New York.

International Symposium on the Biological Activity and Metabolism of Hyaluronan, St. Tropez.

International Symposium, The Clinical Impact of Bone and Connective Tissue Markers, sponsored by Uppsala University and Pharmacia AB.

The Ciba Foundation, London.

Opening Lecture in Session on Hyaluronan, Fourth Gordon

Conference on Proteoglycans, U.S.

Symposium on Glycosaminoglycan Metabolism, Faculty of Medicine & Dentistry, University of Alabama at Birmingham.

The Uppsala BMC Summer Program 1991, Sättra Brunn, Sweden.

1st International Workshop on Hyaluronan in Drug Delivery, U.K. 1993.

Royal Swedish Academy of Sciences, 1993 : Member, invited group of Australian scientists. Lectures & Seminars at National Meeting, and Universities of Uppsala and Lund.

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PRESENT RESEARCH ACTIVITIES:

Biosynthesis and metabolism of hyaluronic acid.

Viscous and other physical studies of synovial fluid and hyaluronan.

Biology and medical significance of hyaluronan.

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EXHIBIT 2

Changes in the relative molecular mass of circulating hyaluronan

J Robert E Fraser

Marc Brown and I have discussed the absorption of hyaluronan by the skin, and have agreed that our group pursue studies in the skin of the Hairless strain of mouse, where skin metabolism, blood flow and lymphatic circulation will be intact, although we cannot expect the same quantitative data. We are currently preparing the relatively massive quantities of purified radioactive hyaluronan required. My remarks will therefore be confined to other facets of hyaluronan turnover presently studied in our laboratories.

One aspect concerns the breakdown of hyaluronan in the bloodstream. Laurent has reviewed the turnover in the peripheral tissues, which obviously varies from one to another. Lymphatic outflow will not contribute much in a tissue like bone, where much of the turnover must be metabolic and on site. The turnover of hyaluronan in cartilage must, to some degree, be a similar story. In soft tissues which are well drained by lymphatics, such as skin and synovium, some 10–35% of the turnover is by local metabolic degradation [1,2] and the remainder goes into lymphatics.

We have been interested in the relative molecular mass (M_r) or molecular weight of hyaluronan in its movement through the body. In the tissues this is mostly in the order of millions, though there is some regional variation. M_r of hyaluronan from the vitreous body of the eye varies with species, age and season [3]. It is large in skin and joints. In normal synovial fluid, the weight-average M_r is in the order of seven million [4]. To our delight we have been able to identify and tap peripheral lymph vessels draining the hock joint in the sheep after injecting labelled hyaluronan of high molecular weight. Laurent found that its M_r in lymph was as high as that of labelled hyaluronan remaining in the synovial cavity. In a joint with acute immunologically-induced arthritis, the hyaluronan polymers in the lymph were much smaller. How do such very large polymers escape from the joint space? I believe they must be simply squeezed or flushed out.

The concentration of hyaluronan in peripheral lymph is usually high relative to that in postnodal lymph or blood and its M_r more or less represents that of hyaluronan in the tissues, or, at least, what is in the more mobile tissue pools. The earlier studies which established the circulation of hyaluronan in lymph [5] were mainly performed on lymph which had passed through lymph nodes, where its molecular weight is distinctly lower than in the tissues [6]. It seemed that perhaps this could be explained by selective escape of small polymers or partial degradation in the tissues, which may well be so.

Eventually we were able to obtain ample amounts of prenodal or peripheral lymph, through the generosity of Ross Cahill and Wayne Kimpton, who are immunologists

primarily interested in the cell and antibody content, whereas I wanted the fluid and its other constituents. Working mainly in sheep, they tap lymph from a variety of sites. The weight-average M_w of hyaluronan is usually several million in lymph before entering the node, but afterwards is reduced to a few hundred thousand or less. The difference was not so great in our one pair of intestinal lymph samples, but there has been no overlap of molecular weight distribution in any of the lymph drainage areas we have examined. Lymphatic hyaluronan is polydisperse, but the same order of difference is found in number-average M_n . We have also found lower M_n in the efferent residue of labelled hyaluronan which escapes absorption and catabolism after perfusion through lymph vessels afferent to a node. Even so, the molecular weight of hyaluronan entering the blood through the thoracic duct is still distinctly higher than of that circulating in blood plasma [6]. Laurent *et al.* have shown that the liver endothelial cell receptors for hyaluronan have a much higher affinity for the larger polymers [7] and it would seem logical that they would be stripped out of the bloodstream and afferent lymph [8] faster.

We examined this phenomenon specifically by studying the elimination of a mixture of hyaluronan of high and low molecular weight from the plasma and simply measuring the proportion above and below 1×10^6 at successive intervals. This appeared to resolve the elimination into a fast phase for the high molecular weight fraction, with $t_{1/2} \sim 1$ min, and a slower phase for the low molecular weight fraction, with $t_{1/2} \sim 7$ min. When we later determined the half-life for large and small polymers separately, we found the latter showed a shorter $t_{1/2}$ in both sheep and rabbits. This was not due to loss in the urine because the molecular dimensions of hyaluronan are so large that a polymer of 20000 Da can scarcely pass through the glomerular capillaries, in contrast to proteins. A full analysis of molecular weight profile in successive plasma samples after injection of large polymers in rabbits showed those of highest molecular weight were nevertheless disappearing first. The elimination of small amounts is very rapid whatever the molecular weight, and there is very little to examine after 2-3 min. At the later intervals, however, there appeared to be slightly more of the smaller polymers than one min after injection (T. Brown & J.R.E. Fraser, unpublished).

When we injected larger amounts to ensure sufficient for analysis at later intervals, it was clear that the polymers remaining in the blood stream were being progressively broken down. We have analysed this phenomenon further since the last meeting (Fraser, Brown & Pierscionek, work in progress). The molecular weight profile of labelled hyaluronan remains stable when incubated with whole blood, fresh plasma or enriched leucocyte fractions in controlled pH ≥ 7.3 . The breakdown is therefore not due to plasma hyaluronidase, which is active only at pH ≤ 5.5 . The next possibility was that there was mechanical shearing in the bloodstream. Enough high-polymer unlabelled hyaluronan (30 mg) was therefore injected before the labelled material to raise plasma viscosity three-fold. This was designed to produce a proportionate reduction in Reynolds number, which determines the critical point for turbulence in blood flow, though we

were not certain that the conditions for turbulence occur in the rabbit's circulation. There was no breakdown of the labelled polymer during 15 min of observation.

This does not exclude polymer reduction by free radicals since hyaluronan absorbs and is broken down by free radical activity, especially the hydroxyl form. It is difficult to envisage a sufficiently high free radical flux in the bloodstream for two reasons. Firstly, there are numerous free radical scavengers in plasma, and secondly, it is difficult to see how the more fragile particulate elements of blood would escape damage at the same time. Nevertheless, we examined this possibility by injecting the same amount of low-polymer unlabelled hyaluronan. This did not slow the breakdown of the large labelled polymers, though its free-radical scavenging activity was calculated to be at least 99% that of the large polymer fraction used previously.

It is probable that some form of mechanical shearing other than turbulence is the cause of this phenomenon. The notion that covalent bonds can be mechanically disrupted meets some initial resistance, but can be readily demonstrated. Another possibility is that hyaluronidase is fixed on cell surfaces somewhere in the circulation which is active \geq pH 7.

We are continuing studies to find a clear explanation. In the meantime, however, these findings are of some relevance to the administration of large amounts of hyaluronan intravenously, especially when high blood levels are to be maintained for long periods. Since the relative viscosity of hyaluronan is exponentially related to its M_v , as well as its concentration, even a modest degree of breakdown in the blood stream will counterbalance the rise in viscosity caused by elevated plasma concentrations.

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EXHIBIT 3

MEMORANDUM
RE: SEIFTER U.K. PATENT 769,287

In the Office Actions, the Examiner primarily relied on Seifter in view of a number of references. What Seifter, U.K. Patent 769,287 does or does not teach becomes paramount to the Patent Office's position. The Examiner has set out his conclusions in the last Official Action after what appears to have been a careful analysis. According to the Examiner, Seifter broadly teaches that hyaluronic acid can be used to facilitate the spreading of a therapeutic agent.

For ease of reference, Applicants set out Applicants' submissions with respect to Seifter in response to the first Official Action sent by the Examiner and the Examiner's response in the latest outstanding action (to which no response has been filed to date). Applicants have underlined portions of their response and portions of the Examiner's response and reasoning for emphasis. Applicants will, in this memorandum, show hereafter, why Applicants believe the Examiner is in error.

Applicants submitted, in the first response to the Official Action in which Seifter first appeared as a reference, as follows:

The main reference relied on by the Examiner is U.K. Patent 769,287. This patent teaches the use of partially depolymerized hyaluronic acid (PDHA) - not hyaluronic acid, salts thereof, fragments or subunits or even homologues, analogues, derivatives, complexes, esters or any other form of hyaluronic acid. The form of hyaluronic acid is partially depolymerized and is termed PDHA. The PDHA is said to be purportedly useful as a spreading agent and lipemia-clearing agent (page 1, left-hand column, lines 14 to 15) and purportedly useful to facilitate the spread and absorption of injected materials in animals and human tissues. (page 1 of patent, left-hand column, lines 37 to 39), (lipemia, Applicants submit, means excessive lipids in the blood). The patent states that this effect is a function of the degree of depolymerization and that it can be employed in drug injections and living tissues (same page, same column, line 39 to 42). Methods are provided which teach the method of manufacture of PDHA by incubation. Longer incubation in the process of manufacture reduces the spreading effect (page 2, left-hand column, lines 8 to 10). PDHA is

compared to hyaluronidase which is also asserted to be an effective spreading agent but is purported to have benefits over hyaluronidase. Spreading does not mean that there is any transport of the agent and penetration. In other words, everything is, as stated, absorbed. Thus, the PDHA is not active in the transport. It is passive. This conclusion is easily recognized by the statement at (page 1, left-hand column, line 38) where the "(PDHA) facilitates the spread and absorption of injected materials". In other words, oil spreads over water but does not penetrate. Material dissolved in the oil could be absorbed into the water. Thus, there would be a depot effect. However, more importantly, this reference is completely irrelevant to hyaluronic acid, pharmaceutically acceptable salts thereof, and subunits and fragments thereof. At page 3, right-hand column, lines 45 to 47, the patent provides:

"Undepolymerized hyaluronic acid (HA) had practically no useful spreading effect as compared with the control of Table II."

It is therefore, clear that this patent teaches away from the use of hyaluronic acid to transport and cause penetration of any Medicine or therapeutic agent. Whatever is meant by the term "spread" in the patent, the word "spread" has no relation to hyaluronic acid. Thus, this patent is irrelevant. This patent provides no motivation for any person skilled in the art to use hyaluronic acid. This reference states that hyaluronic acid has no practical utility in respect of spreading whatever that may mean. (Applicants' position is that spreading has nothing whatsoever to do with transport and penetration.)

The '287 patent was published in 1957.

The Examiner, in the follow-up Official Action (which presently remains unanswered) stated as follows in response to Applicants' above submission and gave his reasoning:

"Firstly, the Examiner contends that the hyaluronic acid and/or pharmaceutically acceptable salts thereof and/or fragments, and/or subunits thereof claimed in the instant invention is in fact the same material referred in the Seifter et al reference. To reiterate, Seifter et al., teach the use of partially depolymerized hyaluronic acid as a carrier to facilitate the spreading of therapeutic agents into animal tissue. Seifter et al., teach the careful control of the amount

of depolymerization clearly illustrating that the ability to facilitate spreading is dependent upon the degree to which the hyaluronic acid is depolymerized. Seifter determines the appropriate form of hyaluronic acid by amount of time used for depolymerization, see page 2, lines 105 to 130.

Applicants correctly point out that Seifter et al., state that un-depolymerized hyaluronic acid had "practically no useful spreading effect", page 3, right hand column, line 46. This was not taken as "teaching away" from the instant invention but, to support the Examiner's position, the Seifter et al., and the instant application are teaching the same composition. The un-depolymerized hyaluronic acid being referred to by Seifter et al., appears to be native, undegraded material extracted from bacteria, which would according to Balazs et al., in the article titled "Hyaluronic acid its structure and use", have a molecular weight of about 1 million, see page 68, left hand column, lines 7-8. Seifter et al., uses hyaluronidase to break the long molecules into shorter molecules, controlling the average molecular size by controlling the length of time the enzyme is allowed to react with the substrate.

Seifter et al., were not able to determine the molecular weight of their material at the time the invention was made, instead, Seifter et al., teach the physical properties of the native un-depolymerized molecule and partially depolymerized molecules, by comparing spreading of dye, contrast media and local anesthesia using the same concentration of hyaluronic acid.

The Examiner contends that Seifter et al., is showing spread in tissue due to differences in the molecular weight of the hyaluronic acid used, with the highest molecular weight (1×10^6) and the lowest weights (oligosaccharides or disaccharides) showing little or no spreading effect, while polymer lengths between the two extremes, for example 50,000 to 750,000, enhance spreading of materials to various degrees depending on the molecular weight. Seifter et al., used the same concentration of hyaluronic acid in both control and test applications so that the only variable was the amount of depolymerization, i.e., molecular weight. The Examiner came to this conclusion by comparing the results of Seifter et al.,

Schultz et al., Della Valle et al., and review such as the Balazs reference. These references, all indicate that hyaluronic acid from natural sources is a very large molecule of $>1-4 \times 10^6$ Daltons, and that the smaller forms (shorter average polymer lengths) of the instant application are most probably the same as the composition obtained by the depolymerization method of Seifter.

Additionally, Seifter, et al., teach at page 4, last two paragraphs, below table VIII; "The terms of hyaluronic acid (HA) and partially depolymerized hyaluronic acid (PDHA) are used in the specification and the claims to include both the free acid and their alkali metal salts. They also include hyaluronic acid and partially depolymerized hyaluronic acid regardless of source of preparation." There is nothing in the Applicants' disclosure that indicates that the product, i.e. purified hyaluronic acid is patentably distinct from the product disclosed by Seifter et al. The Examiner contends that the two products are the same although they may have been obtained by different methods. Applicants are invited to submit evidence providing that the two products are chemically different.

Applicants argue that the PDHA as taught Seifter et al. transports materials by passive means whereas the HA of the instant invention acts by some sort of active transport. Seifter et al., on page 1, left hand column, at lines 30-34, state that "...releases partially depolymerized hyaluronic acid (referred to below as PDHA) which then acts as a transport agent, ion-exchanger, or a protective colloid and peptizing agent, aiding the dispersion of materials into the tissues.", at lines 37-39, "... (PDHA) facilitates the spread and absorption of injected material into animal and human tissues,...". This indicates to the Examiner that Seifter et al., were not sure whether the mechanism involved was active or passive but, that they clearly considered spreading and absorption to be two different properties of their material. Seifter et al., clearly believed that PDHA actually caused or at the very least enabled the spreading of material into the tissues. The Examiner holds that "spreading" is used by Seifter et al., to indicate transport or movement of the material, though the precise means by which this is accomplished, actively or passively, was unknown to Seifter et al.

The instant application does not provide any information which would serve to define the "spreading" of materials by the instant invention over the composition taught in the prior art. This argument is also immaterial, since it is not required that the prior art even know the mechanism by which a composition functions.

Applicants argue that there is no motivation to combine the references taught in the references, i.e., that the motivation to combine the references was derived from the Applicants' disclosure. The Seifter et al., reference broadly teach the use of HA to increase the spreading of therapeutic agents into tissues. Seifter et al, directly provide the motivation to combine their PDHA with a variety of medicinal agents as found in the breadth of their claims and at page 2, left hand column, lines 46-51, wherein they state that the examples are "...illustrative only and not to limit the invention..." and at page 4, left hand column, lines 93-95, "...we have discovered a new product useful in various ways in animal experimentation and in animal and human therapy, and a method of preparing it." These statements clearly teach that the claimed compositions and methods are widely applicable. One skilled in the art would be motivated given the teaching of Seifter et al., to use hyaluronic acid in combination with a plurality of agents to enhance spreading into mammalian tissues. *(Interlineation has been added by Applicants for visibility and emphasis.)*

In Applicants' respectful submission, there is a flaw with respect to the Examiner's reasoning. This flaw was created at the very beginning of the Examiner's reasoning forming the basis of the Patent Office's position. In the most recent action, the Examiner states that Seifter teaches the use of partially depolymerized hyaluronic acid [termed PDHA in the Seifter Patent] as a carrier to facilitate the spreading of therapeutic agents into animal tissue. The Examiner then asserts what he believes Seifter taught:

"Seifter et al. broadly teach that hyaluronic acid can be used to facilitate the spreading of a therapeutic agent, diagnostic agent, x-ray contrast agent, anesthetic agent, and use of hyaluronic acid for parenteral administration."

This is quite a jump from PDHA to hyaluronic acid - when Seifter specifically states at page 3, lines 45-46:

"UNDEPOLYMERIZED HYALURONIC ACID (HA) had practically no useful affect, as compared with the control of Table II."

In the last action , the Examiner made the following statements:

1. "Firstly, the Examiner contends that the hyaluronic acid and/or pharmaceutically acceptable salts thereof and/or fragments and/or subunits thereof claimed in the instant invention is in fact the same material referred in the Seifter et al. reference." (*emphasis added*)
2. "The undepolymerized hyaluronic acid being referred to by Seifter et al., appears to be native, undegraded material extracted from bacteria, which would, according to Balazs et al., in the article titled "Hyaluronic Acid. Its Structure and Use" have a molecular weight of about 1 million, see page 68, left hand column, lines 7-8. Seifter et al., uses hyaluronidase to break the long molecules into shorter molecules, controlling the average molecular size by controlling the length of time the enzyme is allowed to react with the substrate."
3. The Examiner contends that Seifter et al., is showing spread in tissue due to differences in the molecular weight of the hyaluronic acid used, with the highest molecular weight (1×10^6) and the lowest weights (oligosaccharides or disaccharides) showing little or no spreading effect, while polymer lengths between the two extremes, for example, 50,000 to 750,000, enhance spreading of materials to various degrees depending on the molecular weight."

Referring specifically to the Balazs article "Hyaluronic Acid, Its Structure and Use" referred to by the Examiner in 2. above (a copy of which is attached as Schedule "A") and particularly, at page 68, left hand column, lines 7-8, **Balazs does not state the molecular weight** of "native, undegraded material extracted from bacteria" is about one million. What Balazs does state is the following:

"To the author's knowledge, there is no report in the literature of bacterial hyaluronic acid exceeding a molecular weight of approximately one million. It is not known whether the presence of low molecular weight hyaluronic acid in bacterial cultures is due to the action of degrading enzymes (hyaluronidase) which are known to be produced by the same bacteria, to the fact that microorganisms do not produce hyaluronic acid of high molecular weight at all, or to degradation during the preparation process."

From this document alone, it is clear that the Examiner's reasoning is flawed by the assumption that the starting hyaluronic acid material used by Seifter had a molecular weight of one million. (The Balazs article was written in 1984). Therefore, what could have been the molecular weight of the hyaluronic acid used by Seifter et al., (speculation)?

To assist Examiner, Applicants enclose two earlier articles of Torvard Laurent (another well recognized expert in Hyaluronic Acid):

- (a) Studies on Hyaluronic Acid in the Vitreous Body published about 1955 from the Retina Foundation, Department of Ophthalmology, Massachusetts, Eye and Ear Infirmary and Harvard Medical School, Boston, Massachusetts (Schedule "B");

and,

- (b) Structure of Hyaluronic Acid, published in 1970, Chemistry of Hyaluronic Acid In: Chemistry and Molecular Biology of the Intercellular Matrix.. Volumes 1, II, III. Edited by E.A. Balazs. New York: Academic Presss, 1970, pp. 703-732 (Schedule "C").

With respect to (a) Schedule "B", in 1955, Molecular Weights for Hyaluronic Acid derived from umbilical cord had been determined to be 3×10^6 (3,000,000-4,000,000). Thus, in 1955, it was possible to determine approximate

Molecular Weights. (This is contrary to the Examiner's conclusions that molecular weights could not be determined in 1955.)

With respect to (b), **Schedule "C"**, at page 711, Laurent provides a table of published molecular weights of hyaluronic acid derived from different sources as follows:

TABLE III
Some Published Molecular Weights of Hyaluronic Acids from
Different Tissues

Tissue	Molecular Weight	Technique	Reference
Human Umbilical cord	3.4×10^6	Light-scattering	Laurent and Gergely (1955)
Bovine vitreous body	7.7×10^4 1.7×10^6	Light-scattering	Laurent et al. (1960)
Bovine synovial fluid	14×10^6	Light-scattering	Preston et al. (1965)
Human synovial fluid			
Normal	6×10^6	Light-scattering	Balazs, Watson, Duff and Roseman (1967)
Rheumatoid	$(2.7-4.5) \times 10^6$	Light-scattering	
Rooster comb (main fraction)	1.2×10^6	Ultracentrifugation	Swann (1968a)
Streptococcal cultures	0.115×10^6 0.93×10^6	Sedimentation & viscosity	Blumberg, Ogston, Lowther and Rogers (1958)

It is therefore clear that the streptococcal HA (referred to at page 2, line 56 of Seifter et al., U.K. Patent 769,287) could have had a molecular weight between about 115,000 and about 930,000 determined by Blumberg, Ogston, Lowther, and Rogers in 1958 (about the time of Seifter et al.). Thus, the Examiner's contention in 1. above (at page 6 of this Memorandum) that the Seifter et al. PDHA material made by the Seifter et al. process is the same as Applicants' material is not accurate. The Seifter starting hyaluronic acid material may have been, and was in all likelihood, the same as Applicants' hyaluronic material and in this regard, Seifter et al. has concluded that:

**"undepolymerized hyaluronic acid (HA)
had practically no useful spreading
effect..." (page 3, lines 45-46)**

Seifter et al. is therefore irrelevant.

Seifter et al. goes on to provide that umbilical cord HA and Vitreous Human HA can also be depolymerized and used (see page 3, lines 57-58). Laurent, in the Table taken from Chemistry of Hyaluronic Acid (Schedule "C") above states that Human Umbilical cord Hyaluronic Acid has a molecular weight in the order of $3-4 \times 10^6$ consistent with the teachings in Schedule "B" (umbilical cord derived HA has a molecular weight of $3-4 \times 10^6$). Bovine Vitreous Body HA from Laurent (Schedule "C") has a molecular weight form $7.7 \times 10^4 - 1.7 \times 10^6$ (77,000-1,700,000). See also U.S. Patent 4,141,973 at column 1, lines 32-42. (U.S. Patent 4,141,973 is not attached.) Once again, Seifter says the undepolymerized hyaluronic acid (HA) has practically no useful spreading affect (page 3, lines 45-46). It is only when the hyaluronic acid is partially depolymerized that the spreading affect is achieved (irrespective of the molecular weight of the hyaluronic acid used as the starting material which original hyaluronic acid starting material does not work).

The reason, Applicants believe, should now be self-evident. Seifter's partially depolymerized material PDHA still contains hyaluronidase even after autoclaving and precipitation. (It is contaminated.) For example, the only element remaining where a high molecular weight hyaluronic acid (hyaluronic acid which won't spread) is partially depolymerized to a lower molecular weight hyaluronic acid (which causes spreading according to Seifter) which may have the same molecular weight as an undepolymerized hyaluronic acid (HA) starting material (which won't spread - has no useful spreading affect) is hyaluronidase.

Applicants appreciate that Seifter et al. states that the reaction solution is heated to deactivate the hyaluronidase and to precipitate the deactivated hyaluronidase from the solution (see page 1, lines 83-89). However, hyaluronidase remains behind in the solution. [Seifter et al. was published in 1957.] There can be no other explanation.

In this regard, see Schedule "C" at page 704 which provides as follows:

"The isolation of pure hyaluronic acid from tissues has been performed in a variety of ways similar to those used for other polysaccharides. Hyaluronic acid can generally be easily extracted in a high yield using water or salt solutions. The polysaccharides may then be precipitated from the solution with an organic solvent, e.g. ethanol, or a quaternary ammonium compound such as cetylpyridinium chloride (CPC) (Scott, 1960). These precipitation procedures will not, however, remove all extraneous proteins and various methods for making "protein-free" hyaluronic acid have been described, e.g. proteolytic digestion (Scott, 1960), chloroform-isoamylalcohol extraction (Blix and Snellman, 1945), chromatography on ion exchangers and adsorbents (Cifonelli and Mayeda, 1957; Berman, 1962; Preston, Davies and Ogston, 1965; Swann, 1968A), elctrodeposition or elctrophoresis (Roseman, Watson, Duff and Robinson, 1955; Sandson and Hamerman, 1962) and centrifugation in CsCl-gradients (Silpananta, Dunstone and Ogston, 1968). The amount of protein which remains after the purification is usually very small, even when only physical methods have been employed, and amounts as low as 0.2-0.3% have been reported (Swann, 1968b).

(See also U.S. Patent 4,141,973 - the preparation of an ultra-pure high molecular weight hyaluronic acid fraction.)

Thus, Applicants respectfully submit that the spreading allegedly caused by Seifter et al.'s, PDHA is identical to the spreading caused by hyaluronidase because the spreading effect is, in fact, contributed by the remaining hyaluronidase. The Examiner will appreciate that hyaluronidase acts to spread material by the digestion of material between the cells thereby permitting material accompanying the hyaluronidase to spread into that space between the cells.. Applicants liken the spreading effect caused by hyaluronidase to putting "Draino" into a clogged drain to "munch" on the clog to permit the material, namely the water, to pass into the space created by the "Draino". In Applicants' respectful submission, this is clear from the teachings of U.K. Patent 769,287 as a whole. The Examiner refers to page 1, left hand column to the words at lines 31-

32 that PDHA is as a transport agent. However, the Examiner will appreciate on re-examination that the transport being referred to, is the prior art which prior art combines hyaluronidase and partially depolymerized hyaluronic acid by the enzyme. The discussion as a transport agent is merely a discussion of possibilities. It is speculation. This transport agent reference is found in the following:

"This action is usually attributed to the decrease in viscosity of the ground substance which results from depolymerization of the hyaluronate by the enzyme. The effect is to decrease the normal resistance of the barrier. Another possibility is the enzyme releases partially depolymerized hyaluronic acid (referred to below as PDHA) which then acts as a transport agent, ion-exchanger, or a protective colloid and peptizing agent, aiding the dispersion of materials in the tissues."
(See page 1, lines 23-34 of U.K. Patent 769,287 - Seifter.)

It is the basis of Seifter et al., that because the PDHA (partially depolymerized hyaluronic acid) is not antigenic and more stable in solution, it is preferred over hyaluronidase. The PDHA is purportedly isolated from the hyaluronidase by the process outlined at page 1, lines 75-89. However, Applicants believe hyaluronidase remains - the solution is contaminated by hyaluronidase.

It is thus clear that what Seifter et al. thought was the formation of PDHA (without hyaluronidase) was really PDHA with hyaluronidase.

Seifter et al. discusses the partial depolymerization using hyaluronidase stating that such action should take place between 5 minutes and less than 60 minutes (page 2, lines 15-16). "If the depolymerization by the enzyme is prolonged to 60 minutes, the spreading action is lessened by at least 50%" (page 2, lines 80-82). Whatever the timing, the hyaluronidase must be removed (discussed above). And the removal is not as simple as autoclaving and precipitating. See Laurent - Structure of Hyaluronic Acid (Schedule "C") at page 704. And that was Seifter et al.'s downfall. The resultant depolymerized product had a spreading action like hyaluronidase but did not have any spreading action if the partial depolymerization did not take place ("Undepolymerized hyaluronic acid (HA) had practically no useful spreading affect...", page 3, lines 45-46) irrespective of the starting Hyaluronic Acid (Streptococcal HA, Umbilical Cord

HA, Vitreous Humour HA, page 3, lines 56-58). As stated before, if the HA is partially depolymerized by hyaluronidase, it spread. It may have been the same molecular weight as the HA that is not depolymerized. (Umbilical Cord HA may have been partially depolymerized to the same molecular weight as streptococcal HA which has not been partially depolymerized - in that event the Umbilical Cord HA which has been partially depolymerized will spread but the streptococcal HA which has not been partially depolymerized won't spread even though both are of the same molecular weight.)

Finally, the Examiner will appreciate that where a molecular weight is specified in the literature with respect to a given specimen of hyaluronic acid, the specimen does not possess a homogenous molecular weight - the specimen is not unimolecular. The molecular weight of the specimen is an average molecular weight determined having regard to the range of molecular weights of the hyaluronic acid present in the specimen. The amounts of the individual molecular weights of the hyaluronic acid in the specimen would (if graphed) appear distributed under a "bell curve" plotted having regard to molecular weight v. amount of that molecular weight present in the specimen. Thus, for example some of the specimen of hyaluronic acid having an "average" molecular weight of about 1,000,000 (referred to by the Examiner and discussed at page 6, (para. 2) of this memorandum), would have a molecular weight less than 750,000 daltons. In that event, the hyaluronic acid used by Seifter et al., should provide some spreading effect (based on the Examiner's reasoning because of the presence of this lower molecular weight hyaluronic acid before partial depolymerization - at a time there had been no partial depolymerization). However, to repeat Seifter et al., at page 3, lines 45-46:

"UNDEPOLYMERIZED HYALURONIC ACID (HA) had practically no useful affect, as compared with the control of Table II."

Thus, as stated above, the hyaluronidase remaining after autoclaving and precipitation must be the reason for the difference. Thus, there is no recognition/teaching by Seifter et al., of transportation by Hyaluronic Acid. Seifter et al. is thus irrelevant, teaching away from Applicants' invention herein.